NORMAN workshop

Integrated Exposure and Effects Assessment

11-12 April 2017

Amsterdam, The Netherlands

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Abstract book

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Network of reference laboratories for monitoring of emerging environmental pollutants

VRIJE UNIVERSITEIT AMSTERDAM

Norman workshop
Integrated Exposure and Effects Assessment

Workshop programme

Tuesday 11 April 2017

Chemical Analysis and Effect Assessment

9:00 Registration, coffee & tea, poster mounting
9:30 Welcome address (Marja Lamoree, VU-E&H)

Chair: Pim Leonards

9:45 **Keynote Ann-Sofie Wernersson**, Swedish Agency for Marine and Water Management:
Effect based tools in a water and marine regulatory framework - current use and future prospects

10:25 Nicole Munz, EAWAG:
Combining chemical analysis, bioanalysis and risk assessment to prioritize risk driving substances in wastewater-impacted streams

10:45 *Coffee break*

11:15 **Keynote Marc Mills**, US EPA, National Risk Management Research Laboratory, Cincinnati:
Using Weight of Evidence to conduct an integrated exposure and effects assessment of contaminant sources

11:55 Corine Houtman, The Water Laboratory:
Integrated analytical approaches to assess chemicals of concern in the water cycle

12:15 Annemarie van Wezel, KWR Water:
Chemical and biological assessment, application to unconventional tight sand gas related waters and in the context of the Dutch signaling value for 'other antropogenic substances'

12:35 *Lunch and poster session*

Chair: Jessica Legradi

13:45 Milo de Baat, UvA-IBED:
Nationwide screening of herbicide risk to algae

14:05 Ron van der Oost, Waternet:
SIMONI: Smart Integrated MONItoring strategy for bioanalytical water quality assessment

14:25 Timo Hamers, VU-E&H:
TIPTOP: Time Integrative Passive sampling combined with TOxicity Profiling - an effect-based strategy for cost-effective chemical water quality assessment

14:45 *Coffee Break*

15:15 Kirsten Baken, KWR Water:
Tracing nitrogenous disinfection byproducts after medium pressure UV water treatment by stable isotope labeling, high resolution mass spectrometry and effect directed analysis

15:35 Nick Zwart, VU-E&H:
Development of a high resolution Effect-Directed Analysis platform for the identification of mutagens and endocrine disruptors in the aquatic environment

15:55 Wrap up and closing for the day; directions for the dinner event

16:15 **End of first day**

Wednesday 12 April 2017

Effect Assessment including Omics Techniques

9:00 Coffee & tea

Chair: Timo Hamers

9:30 **Keynote Go Suzuki**, National Institute for Environmental Studies, Japan:
Chemical safety assessment using an integrated exposure and effect analysis

10:10 Peter Behnisch, BioDetection Systems:
In vitro effect based toxicity profiling and water monitoring

10:30 Sebastian Buchinger, German Federal Institute for Hydrology:
Screening approach for compounds in surface- and wastewater samples exhibiting estrogenic potentials

10:50 *Coffee break*

11:20 **Keynote François Brion**, INERIS, France:
Integrated approaches to investigate the effect of progestins in fish and their occurrence in the aquatic environment

12:00 Hélène Arambourou, Irstea:
Effects of exposure to an analog of the juvenile hormone on the freshwater amphipod Gammarus fossarum

12:20 Cornelia Kienle, Swiss Centre for Applied Ecotoxicology Eawag-EPFL:
The Combined Algae Assay - a promising tool for water quality assessment

12:40 *Lunch, poster session and demo of SPARK Integrity spotter*

Chair: Selim Aït-Aïssa

14:00 Dimitrios Damalas, National and Kapodistrian University of Athens:
From toxicity assay to metabolomics analysis - An integrated approach to assess the toxicity of three benzotriazoles in zebrafish (Danio rerio) embryos

14:20 Jessica Legradi, VU-E&H:
Application of novel omics tools for zebrafish (neuro-)toxicological research

14:40 Closing and farewell

15:00 End of the workshop

Venue
Auditorium of the O|2 Building
Vrije Universiteit
De Boelelaan 1108
1081 HZ Amsterdam
The Netherlands
The venue can be easily reached by train (Amsterdam Zuid)
Tram and metro lines that stop within walking distance of the meeting venue:
Tram 5, metro 51: stop De Boelelaan/VU
Tram 16: stop VU medisch centrum
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Keynotes

EFFECT BASED TOOLS IN A WATER AND MARINE REGULATORY FRAMEWORK – CURRENT USE AND FUTURE PROSPECTS

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The current assessment of risks from chemicals in and via the aquatic environment in the Water Framework Directive (WFD) context is based on a substance by substance approach, including the establishment of environmental quality standards (EQS) on European and member state levels[1].

In the 2010-2012 Work Program of the Common Implementation Strategy (CIS) for the WFD, the subgroup on Chemical Monitoring and Emerging Pollutants (CMEP) under the WG "Chemical Aspects" received a mandate for the elaboration of a technical report on effect-based tools (EBT) applicable to surface water monitoring. The activity progressively involved several Member States, JRC, associated States and stakeholders in an EU-wide drafting group (47 Experts).

The resulting technical report (Wernersson et al. 2014) identified and described several EBTs (e.g. bioassays, biomarkers and new ecological indicators) and objectives for using EBTs in the current WFD context, including:

- as part of the pressures and impacts assessment to aid in the prioritisation of monitoring activities
- to establish early warning systems
- to provide additional support in water and sediment quality assessment.

Furthermore, EBTs can be applied in the Marine Strategy Framework Directive (MSFD) context and the report also includes a specific chapter on current marine use.

In November 2016 the European Commission presented a proposal to the Water Directors, related to the WFD review, suggesting a more holistic approach, taking into account the presence of mixtures of chemicals acting together (for example through the use of EBTs in addition to group EQSs). The purpose is to provide a more accurate assessment of risks and a more appropriate targeting of monitoring and measures.

As part of the CIS work program for 2016-2018 a new activity on EBTs is starting up. Its main objective is to examine and further document the possible implementation of EBTs for monitoring and assessment in the WFD context, alongside traditional chemical analysis, bearing in mind possible application under the MSFD. The activity will be linked to the previous report and past and ongoing projects. It will focus on how EBTs can be used alongside chemical analytical approaches. Practical implementation issues such as routine feasibility and costs as well as approaches to identify and implement measures to reduce emissions are some of the aspects that will need to be considered. The activity is expected to deliver short- and long term recommendations by 2018.

The technical report and current CIS activity on EBTs and link with NORMAN WG 2 will be presented and discussed.

[1] European EQSs are regulated by 2008/105/EC (revised by 2013/39/EU). On national level, Member States establish national EQSs for "River basin specific pollutants" (RBSPs).

Chemical Safety Assessment Using an Integrated Exposure and Effect Analysis: Case Study for Oligomeric and Polymeric Flame Retardants

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Flame retardants (FRs) are widely used for household items such as televisions, computers, fabrics and flameproof curtain as additives to prevent the spread of fire. FRs can be divided into inorganic and organic FRs. There are two of the major groups of organic FRs that are halogenated FRs including brominated and chlorinated FRs (BFRs and CFRs, respectively), and phosphorus-containing FRs (PFRs). The use of organic FRs is of concern in the environmental contamination issue especially in the intervening quarter-century. Based on cumulated research evidence about suspicion of toxicity and findings in the environment and biota, a part of polybrominated diphenylethers and 1,2,5,6,9,10-hexabromocyclododecane used as monomeric BFRs have been included in Stockholm Convention on Persistent Organic Pollutants (POPs) in 2009 and 2012 for total ban or limitation on their production and use (POP-BFRs). This is for reasons of potentially harmful effects to human and wildlife health based on information in relation to their persistence, bioaccumulation, toxicity and long-range transport. However, the restrictions and/or limitations on production and use of POP-BFRs have led to increased use of alternative FRs. Recently, CFRs and PFRs have also been detected in indoor dust as an excellent indicator of chemical exposure in indoor environment. Their concentrations in indoor dust were also in the order of micrograms per gram, which are equivalent to or exceed those of POP-BFRs. *In vitro* endocrine-disrupting potency for monomeric CFRs and PFRs has also been reported by several studies up to now. On the basis of these situation, there is an urgent need to provide the design concept concerning chemical safety for FRs. As part of the chemical safety assessment leading to more proper design, this study has investigated *in vitro* endocrine-disrupting potency of oligomeric and polymeric FRs such as resorcinol bis(diphenyl phosphate) (PBDPP), resorcinol bis[di(2,6-dimethylphenyl) phosphate] (PBDMPP) and bisphenol A bis(diphenyl phosphate) (BPA-BDPP), and diethylene glycol bis[di(2-chloroisopropyl) phosphate] (DEG-BDCIPP) by using a panel of human-cell–based CALUX reporter gene assays to evaluate steroid-hormone–disrupting potency [that is, human androgen receptor (AR), estrogen receptor α (ERα), progesterone receptor (PR), and glucocorticoid receptor (GR) -mediated activities], lipid metabolism and immune function–disrupting potency [human peroxisome proliferator–activated receptor γ2 (PPARγ2) -mediated activity] as markers of toxicity. Preliminary results indicated that the oligomeric and polymeric FRs containing impurities had *in vitro* endocrine-disrupting potencies as ERα agonism and AR, PR and GR antagonism. Further bioassay-directed chemical analysis for ERα agonistic potency tended to reveal that their impurities in connection with monomeric FRs and their related compounds, but not main oligomeric and polymeric agents, strongly contribute to these activities. We concluded that bulky FRs such as oligomeric and polymeric types would exert no or only a low potency, due to steric hindrance in cellular uptake and nuclear receptor binding pockets. Our results suggest that oligomeric and polymeric design for FRs are one of proper chemical designs providing safety and management of impurities is of particular importance from an *in vitro* toxicological point of view.

Integrated approaches to investigate the effect of progestins in fish and their occurrence in the aquatic environment

François Brion[1], Sélim Aït-Aïssa[1], Olivier Kah[2], Patrick Balaguer[3], Budzinski Hélène[4]

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To date, most studies on endocrine disrupting compounds have focused on estrogenic ones with a particular attention to natural (estradiol, estrone, estriol) and synthetic estrogens (17α-ethinylestradiol). However, there are now emerging evidences on the occurrence of other natural and synthetic steroids in aquatic systems. Among them, the potential risk on aquatic species posed by synthetic progestins, widely used as oral contraceptive and hormone replacement therapy, has been recently pointed out. However, their occurrence, fate and effects have been poorly investigated. In the present study, we used an integrated approach that combines effect-based tools and chemical analyses i) to investigate the cellular mode of action of a broad range of progestins and their effects in zebrafish, ii) to detect (anti)-progestagenic activities in French aquatic systems, and iii) to identify the compounds responsible for (anti)-progestagenic activities.

Twenty-four selected progestins were screened using dedicated reporter gene assays for their capacities to interact with human (h) or zebrafish (zf) nuclear receptors (PR, ER, AR, GR) *in vitro* and to alter the cell-specific expression of target genes in zebrafish embryos *in vivo*. By using human reporter cell lines stably expressing either hPR or zfPR, we revealed marked interspecies differences on the ability of progestins to activate/antagonize PRs. We also demonstrated that progestins can interfere with multiple NR signaling pathways *in vitro* eliciting estrogenic, androgenic and/or (anti)glucorticoid activities, in a species-specific manner. Their capacities to interfere with hormone-regulated genes were further evaluated *in vivo* in zebrafish. In transgenic cyp19a1b-GFP embryos, progestins derived from 19-nor-testosterone induced estrogenic responses in radial glial cells through an ER-dependent mechanism. Some of them also disrupted GR-signaling pathway in transgenic cyp11c1-GFP larvae. Altogether, our data show complex toxicological profiles of progestins and their capacities to interfere with the biosynthesis of neuro-estrogens and corticosteroids in the developing fish.

To assess the potential exposure of aquatic organisms to ligands of the progesterone receptor, a bioanalytical approach was used to detect and quantify progestagenic activities in environmental samples (including wastewater, surface waters and sediments). For the first time, h and zf (anti)progestagenic activities in aquatic samples were reported showing wastewaters as important sources of contamination. Importantly, marked interspecies differences were highlighted between h and zf with noticeable zebrafish-specific PR agonistic activities quantified at several sites. To identify zebrafish-specific PR active compounds at these sites, environmental extracted were fractionated using RP-HPLC and several zfPR- but not hPR-active fractions were isolated, hence confirming fish-specificity. Non-target chemical analysis using LC-Q-TOF in these fractions allowed identification of steroidal structures and pharmaceuticals. Their biological activities on zfPR and hPR will be tested.

Overall our project provides an extensive (eco)toxicological characterization of currently used progestin pharmaceuticals on key molecular endocrine targets. It also depicted for the first time (anti)progestagenic activities in aquatic samples showing wastewaters as a source of contamination by zf-active compounds, some of which have been identified. Overall, this study supports the need to further characterize ecotoxicological hazards and risks posed by progestins.

Keywords: progestins, hazard, *in vitro*, *in vivo*, zebrafish, bioanalysis.

Oral presentations

COMBINING CHEMICAL ANALYSIS, BIOANALYIS AND RISK ASSESSMENT TO PRIORITIZE RISK DRIVING SUBSTANCES IN WASTEWATER-IMPACTED STREAMS

Nicole A. Munz[1,2], Francis J. Burdon[1], Dick de Zwart[3], Beate Escher[4,5,6,7], Peta A. Neale[6,7], Heinz P. Singer[1], Christian Stamm[1], Juliane Hollender[1,2]

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Wastewater treatment plants (WWTP) present a major source of micropollutants to the aquatic environment. Aquatic organisms are therefore constantly exposed to chemical mixtures, which can impose negative impacts on the ecosystem. In this study, we investigated concentration patterns of a large number of micropollutants in wastewater-impacted streams, as well as in the effluents, across independent catchments with different land uses during low flow conditions. Acute risk was predicted using the multi-substance potentially affected fraction approach and compared with biomonitoring data using the SPEAR index indicative of pesticide sensitivity. Grab samples were taken at 24 Swiss WWTPs (effluent, upstream, downstream) during eight time points and analyzed for almost 400 organic substances. Besides pharmaceuticals and other typical household chemicals, also many pesticides were included, as we wanted to investigate whether the higher loaded pharmaceuticals or the episodically discharged pesticides - when released during low flow conditions - contribute most to the risk towards aquatic organisms. Macroinvertebrate data was collected at the same sites during two time points in spring. In a parallel study a battery of 13 ecotoxicological bioassays was conducted at three selected sites and mixture toxicity modeling was performed to assess the contribution of the detected chemicals to the observed effects. As expected, a multitude of micropollutants was regularly detected and the concentrations were mostly higher downstream than upstream. Further, a positive correlation of plant protection products upstream and arable land use could be observed. While pharmaceuticals and other typical household chemicals were regularly detected in the effluents, many pesticides were detected only during episodic events and are thus underrepresented with grab samples. Nevertheless, occasional concentration peaks were observed for pesticides in the stream as well as in the effluents and the acute toxic pressure was mainly driven by pesticides. The lack of effect data for pharmaceuticals limits, however, interpretation for this substance group. Overall, rather low acute risk was predicted ranging from 0% to 2.1% of affected species over all sites and time points with only a few substances – mainly pesticides and diclofenac - explaining already the total risk. The mixture toxicity modelling combining chemical analysis and bioanalysis conducted at three sites underlined the relevance of single substances as drivers of toxicity. On the other hand, the lack of effect data was a limiting factor for the evaluation of several bioassays and in many cases only a small fraction of the effect was explained by the detected chemicals indicating a joint effect of many unknown substances. Overall, despite the low predicted risk a significant positive correlation with the SPEAR index was observed, highlighting the importance of pesticides in wastewater-impacted streams. This relevance of pesticides also during low flow conditions seems to be typical for catchments where urban and agricultural land use co-occur as it is the case for many European countries.

Munz, N. A., Burdon, F. J., de Zwart, D., Junghans, M., Melo, L., Reyes, M., Schönenberger, U., Singer, H. P., Spycher, B., Hollender, J., & Stamm, C., 2017. Pesticides drive risk of micropollutants in wastewater-impacted streams during low flow conditions. Water Res, 110, 366-377.

Neale, P. A., Munz, N. A., Ait-Aissa, S., Altenburger, R., Brion, F., Busch, W., Escher, B. I., Hilscherova, K., Kienle, C., Novak, J., Seiler, T. B., Shao, Y., Stamm, C., & Hollender, J., 2017. Integrating chemical analysis and bioanalysis to evaluate the contribution of wastewater effluent on the micropollutant burden in small streams. STOTEN, 576, 785-795.

INTEGRATED ANALYTICAL APPROACHES TO ASSES CHEMICALS OF CONCERN (CEC) IN THE WATER CYCLE

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Our modern society uses thousands of different organic compounds that end up in the water cycle. This is worrying, as these compounds might adversely affect public (via drinking water) and ecosystem health. Therefore, monitoring and risk assessment of CECs in the water cycle is of vital importance in order to take necessary measures. Nowadays, a paradigm shift is taking place in which more and more in vitro bioassays and screening techniques are used to this purpose instead of only chemical target analyses. This presentation discusses two current studies on approaches to investigate the presence of bioactive compounds in the water cycle, using in vitro bioassays as a screening tool in combination with chemico-analytical techniques.

In the first study the fate of hormones in four waste water treatment plants was investigated. A panel of in vitro reporter gene CALUX® bioassays was used for glucocorticoid, estrogenic, (anti-)androgenic and progestagenic compounds. This was combined with sensitive multi-component methods for hormones on UPLC-tQ-MS. Estrogenic and anti-androgenic activity appeared to be removed well in the treatment. Anti-androgenic activity, on the other hand, appeared to be formed during treatment as it was found only in effluent samples. Glucocorticoid and progestagenic activity were found in influent as well as effluent samples, indicating no removal. Twelve different hormones were identified in the samples. Glucocorticoid activity in influent could be explained by the presence of dexamethasone, triamcinoleacetonide, prednicarbate and amcinonide. However the glucocorticoid activity in effluent could only for a small part be explained by compounds in our target analysis, indicating the formation of unknown active metabolites. Therefore, as a follow-up study a newly developed Effect-directed Analysis Platform was applied to provide more insight in the identity of the active compounds using high resolution MS screening. Principal Component Analysis of the analysis results of the hormones, bioassays and analysis data of other contaminants in the same samples proved very helpful to profile the WWTPs and treatment steps according to their pattern of contaminants.

National and European Drinking Water Legislations oblige drinking water companies to monitor 11 (EU: 4) of the 16 EPA PAHs and seven so called indicator PCBs (national legislation) in drinking water. However, current monitoring – target analysis by GC-MS – has a couple of limitations. The congeners mentioned in the law are not necessarily those with the highest toxicity or the highest presence in water. In addition, other congeners or other relevant compounds with similar toxicities present in water but not mentioned in the law are overlooked. In the second study, we therefore investigated the suitability of bioassays as an alternative monitoring tool for these compounds. Based on current knowledge on the mechanisms of action of PCB and PAH toxicity, a panel of four CALUX bioassays (DR-, PAH-, PXR-, and anti-AR-CALUX) were selected. Pure standards of individual compounds and mixtures were tested in the assays to assess their activity. In addition, a water sample intentionally for this project enriched with coal tar (bitumen) coated piping was tested as a worst case example of a field sample. The study provided us with new data of toxic activities of compounds by various mechanisms. It indicated that especially anti-AR CALUX and PAH-CALUX could provide valuable tools for a more risk based monitoring of compounds with PAH- and PCB-like toxicities in the water cycle instead of the now used target analysis.

Chemical and biological assessment, application to unconventional tight sand gas related waters and in the context of the Dutch signaling value for 'other antropogenic substances'

Annemarie P. van Wezel[ab] , Ann-Hélène Faber[abc*], Mark P.J.A. Annevelink[a*], Kirsten Baken[a]

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ABSTRACT

Bioanalytical tools hold great promise as an additional tool of our current water monitoring strategies. In vitro bioassays are increasingly applied in water quality assessment, and able to specify and quantify measure early adverse effects of contaminants in water including a measure of mixture effect in low doses. Recent large scale projects delivered several methodological advances leading to a comprehensive framework including the most promising panel of assays and expanded effect-based trigger values (EBT) for both drinking water and environmental waters (GWRC Endocrine Toolbox II, ITN EDA-Emerge, FP7 DEMEAU, FP7 Solutions). These innovations could contribute to strengthen the safety of conventional water treatment plants and be integrated in future regulations. A critical next step will be to derive further trigger values for an expanded scope of bioassay endpoints. Several strategies have been proposed to this end, but further acceptance and harmonization will be needed to the end of integration into water quality and safety frameworks and legislation.

Here, we present the use of bioassays in the context of evaluation of unconventional tight sand gas related waters. Chemical risk assessment of hydraulic fracturing is generally based on shale gas related practices in the U.S., lacking other types of gas development also using hydraulic fracturing. Here, we focus on relatively polar organics present in hydraulic fracturing related waters from a tight sand gas development in the Netherlands. Fracturing fluid, flowback water samples and surrounding aquifers before and after the actual fracturing were analysed by means of HR LC-MS/MS, the Ames test and several CALUX bioassays.

A suspect list (candidate compounds) containing 881 chemicals was based on US and EU used and produced chemicals related to hydraulic fracturing. Less than half of these global candidate compounds are currently registered under European legislation. Considering that hydraulic fracturing in Europe only can make use of authorized chemicals, the amount of possible chemicals is restricted compared to the US. In the fracturing fluid samples, 1009 different compounds are detected, including 11 that matched with the suspect list. 714 of these occur in concentrations – semi-quantitatively expressed as internal standard equivalent - exceeding groundwater thresholds based on the Threshold of Toxicological Concern (TTC) of 0.1 µg/L. 348 of these compounds are also retrieved in the flowback samples although at lower concentrations. In the flowback samples a total of 980 peaks were detected, of which 631 originate from the subsurface and 20 could be matched with the candidate list. Between the first and eighth day of flowback, the number of compounds exceeding the TTC value drops from 291 to 189. In the groundwater samples there is no significant change in composition between the samples taken before and after the actual fracturing possibly related to earlier activities at the site, however there is a relation with distance from the well. 50 peaks were detected with 12 exceeding TTC values. The Ames fluctuation test showed genotoxicity for different flowback and fracturing fluid samples. Furthermore, a selection of CALUX bioassay gave positive responses related to oxidative stress and P53 activity for fracturing fluids and flowback. Results point to the importance of the currently in place extensive measures related to the handling, transport and treatment of hydraulic fracturing related waters to avoid adverse environmental and human health impacts.

Furthermore, we will show some results on the use of bioassays and chemical sum parameters in the context of the Dutch signaling value for 'other antropogenic substances', as implementation of both the Water Framework Directive and the Drinking Water Directive.

Nationwide Screening of Herbicide Risk to Algae

de Baat, M.L.[1], Bas, D.A.[1], van Beusekom, S.A.M.[1], van der Meer, F.[2], de Vries, M.[2], van der Oost, R.[3], Verdonschot, P.F.M.[1], Kraak, M.H.S.[1]

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According to the European Union Water Framework Directive (WFD), chemical water quality is assessed by monitoring 45 priority compounds. This list is outdated however, as the selected compounds are not representative of present day contamination. Consequently, a large portion of toxic effects observed in surface waters can often not be attributed to compounds measured by the water authorities. Hence, there is a need for a more effect based monitoring strategy that employs bioassays to identify environmental risk. An adequate selection of bioassays is crucial for this monitoring strategy, as identification of compounds causing the observed effects depends largely on this selection. Algal photosynthesis is a sensitive and well-studied process that enables identification of the presence of hazardous herbicide concentrations in surface water. Therefore the aim of this study was to develop and apply an innovative algal photosynthesis bioassay to assess surface water herbicide risk to algae. To this purpose, *Pseudokirchneriella subcapitata* was exposed to surface water samples in 96-well plates. After 4.5 hours, effective photosystem II efficiency (ΦPSII) was determined using Pulse Amplitude Modulation fluorometry connected to a robot, resulting in a rapid high throughput bioassay. Potential effects of surface water from 39 locations were assessed. Algal photosynthesis was affected by surface water from only one location. Suspect Target Analysis (STA) of this single toxic sample revealed that three herbicides were present above environmental quality standard (EQS) concentrations. The observed effect could largely be attributed to one of these herbicides, linuron, which occurred at 110 times the EQS concentration and which is not included in the WFD priority compounds list. In conclusion, applying the algal photosynthesis bioassay may avoid redundant chemical analyses, while simultaneously identifying the presence of hazardous compounds that would have been overlooked by routine chemical WFD monitoring.

Key words: Algal bioassay, PAM fluorescence, Herbicides, Surface Water Toxicity

SIMONI: SMART INTEGRATED MONITORING STRATEGY FOR BIOANALYTICAL WATER QUALITY ASSESSMENT

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Presentation preference: PLATFORM

Abstract
At the Waternet Institute for the Urban Water Cycle an effect-based chemical monitoring strategy has been developed over the last five years. The objective of this SIMONI strategy (Smart Integrated Monitoring) is to get more information on the chemical water quality for less money than traditional methods (e.g. Water Framework Directive). This project, that bridges the gap between scientific research and regular field monitoring, combines passive sampling with standardized *in situ*, *in vivo* and *in vitro* bioassays. Design and results of the two-tiered SIMONI model for classifying microchemical risks of surface waters will be demonstrated. A simple 'Toxicity traffic light' is developed for policymakers and regulators, indicating low, potential and high risks of organic micropollutants for the ecosystem. Tier 1 of the model is a hazard identification that makes the distinction between low and potential ecological risks. Hazards of organic chemicals are determined with a suite of reliable, fast, user-friendly and inexpensive bioassays. The selection of endpoints for toxicity profiling was directed towards the indication of risks from a broad spectrum of chemical micropollutants to aquatic organisms (e.g. nonspecific toxicity, endocrine disruption, antibiotics activity, genotoxicity, oxidative stress, dioxin-like toxicity). In order to indicate ecological risks, effect-based trigger values (EBT) were designed for all relevant bioassay responses. A three-step approach was used for EBT development, aquatic toxicity data search, species sensitivity distribution of bioanalytical equivalents (BEQ), and benchmark field studies on background responses. Measured bioassay data were incorporated into a simple model that compares the responses with their EBTs and adds a weight factor to different types of bioassays, thus generating a quantitative hazard classification of the entire mixture of micropollutants. Results of field monitoring studies demonstrated the feasibility of this strategy for identifying hot-spots of chemical pollution. It appeared that highest ecological risks occurred at the agricultural greenhouse areas, most likely due to pesticide emissions, while lower risks were observed in waters receiving wwtp effluents, sewage overflows and runoff from landfills. Due to low costs and high relevance, this model has the potential to become the first bioanalytical strategy to be applied in routine water quality monitoring.

Key words: toxicity profiling, bioassays, passive sampling, environmental risk assessment

TIPTOP: **T**IME **I**NTEGRATIVE **P**ASSIVE SAMPLING COMBINED WITH **TO**XICITY **P**ROFILING – AN EFFECT-BASED STRATEGY FOR COST-EFFECTIVE CHEMICAL WATER QUALITY ASSESSMENT

Timo Hamers[a], Foppe Smedes[b,c], Jasperien de Weert[b], Evert-Jan van den Brandhof[d], Dik van de Meent[d,e], Dick de Zwart[d,f]

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The TIPTOP project hypothesizes that time-integrative passive sampling followed by toxicity profiling is a toxicologically and ecologically more relevant and more protective approach for chemical water quality assessment than the assessment based on a comparison between concentrations of predefined individual compounds to their Environmental Quality Standard (EQS). To test this hypothesis, a demonstration study was designed in which passive samplers were deployed for six weeks at eight sampling sites, i.e. at six WFD sites in the Dutch river delta and in two WWTP effluent streams. All eight sampling sites are well-characterized: the WFD sites are biweekly or monthly monitored for surface water pollutants by Rijkswaterstaat, and the two WWTPs are monitored in four-year cycles for priority pollutant emissions within the framework of the European Pollutant Release Transfer Register (E-PRTR). Two types of passive samplers were deployed at each sampling site, i.e. partitioning-based silicone-rubber (SR) samplers and adsorption based Speedisk (SD) samplers. Toxicity profiles of the passive sampler extracts were determined using a test battery consisting of seven *in vitro* bioassays representing different mechanisms of action and six small volume *in vivo* bioassays with species representing different trophic levels. Passive sampler extracts were also chemically analyzed for a selected set of target compounds and for the total molar sum of accumulated compounds. Seven different strategies have been worked out to interpret the bioassay results and the supporting chemical analytical data in terms of risk assessment, i.e.:

1. Benchmark toxicity profiles of surface water to WWTP effluent;
2. Compare water concentrations to EQS;
3. Compare *in vitro* bioassay results to “trigger values”;
4. Determine toxic pressure based on measured concentrations;
5. Determine toxic pressure based on *in vivo* bioassay results;
6. Determine toxic pressure for narcotic compounds;
7. Determine toxic pressure based on specific *in vitro* bioassay results.

Since both the surface water and WWTP effluent samples appeared to be relatively clean, all strategies indicated little to negligible risk from chemical substances, thereby hampering the demonstration of the added value of effect-based monitoring. Therefore, follow-up studies should be performed in relatively small water ways affected by local land use. Still, the added value of effect-based monitoring was clearly pointed by the estimated “distance” of the actual situation in the field towards toxicity threshold values (i.e. margin of exposure). Such information could not be retrieved from numerous chemical analyses below the limit of detection. Moreover, the different strategies made clear that effect-based monitoring is a very useful tool for prioritizing locations for further research (for instance via effect-directed analysis) and/or regulation.

TRACING NITROGENOUS DISINFECTION BYPRODUCTS AFTER MEDIUM PRESSURE UV WATER TREATMENT BY STABLE ISOTOPE LABELING, HIGH RESOLUTION MASS SPECTROMETRY AND EFFECT DIRECTED ANALYSIS

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Advanced oxidation processes are important barriers for organic micropollutants (e.g., pharmaceuticals, pesticides) in (drinking) water treatment. Studies indicate that medium pressure (MP) UV/H_2O_2 treatment leads to a positive response in Ames mutagenicity tests, which is then removed after granulated activated carbon (GAC) filtration. The formed potentially mutagenic substances were hitherto not identified and may result from the reaction of photolysis products of nitrate with (photolysis products of) natural organic material (NOM). In this study we present an innovative approach to trace the formation of disinfection byproducts (DBPs) of MP UV water treatment, based on stable isotope labeled nitrate combined with high resolution mass spectrometry. It was shown that after MP UV treatment of artificial water containing NOM and nitrate, multiple nitrogen containing substances were formed. In total 84 N-DBPs were detected at individual concentrations between 1 to 135 ng/L bentazon-d6 equivalents, with a summed concentration of 1.2 µg/L bentazon-d6 equivalents. For 14 byproducts the structure of the N-DPB was elucidated using in silico fragmentation tools and confirmed with analytical reference standards.
Screening for the 84 N-DBPs in water samples from a full-scale drinking water treatment plant based on MP UV/H_2O_2 treatment showed that 22 of the N-DBPs found in artificial water were also detected in real water samples. In these samples, both chemical analysis and the Ames fluctuation test showed an increased response after MP UV/H_2O_2 treatment.

Further identification of the detected N-DBPs, using effect directed analysis to pinpoint the source of the mutagenicity or individual testing of these substances in Ames tests, will provide more insight into the relation of the N-DBPs with the observed mutagenicity. To this end, fractions of MP UV treated and untreated water extracts were prepared using preparative HPLC. These fractions were each concentrated and tested in the Ames fluctuation test. In addition, high resolution mass spectrometry was performed in all fractions to assess the presence of N-DBPs. After evaluating the results, a correlation was observed in fractionated MP UV treated water samples between the detection of byproducts and detection of mutagenicity. Based on toxicity data and QSAR analysis, we could indicate five N-DBPs that are potentially genotoxic and were present in relatively high concentrations in the fractions in which mutagenicity was observed.

The results of this study offer opportunities to further evaluate the identity, potential health concern and relevance for full scale drinking water treatment plants and varying process conditions of N-DBPs formed during MP UV drinking water treatment.

DEVELOPMENT OF A HIGH-RESOLUTION EFFECT-DIRECTED ANALYSIS PLATFORM FOR IDENTIFICATION OF MUTAGENS AND ENDOCRINE DISRUPTORS IN THE AQUATIC ENVIRONMENT

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Effect-directed analysis is a strategy used for the identification of (unknown) bioactive compounds in mixtures. Fractions from chromatographically separated samples are collected and separately tested in bioassays. Masses in active fractions, from mass spectra recorded in parallel, are analyzed to identify the compound responsible for the observed biological effect. For analysis of complex environmental mixtures like water samples, however, fractionation and bioassay testing used in effect-directed analysis suffer from lack of resolution. The large number of compounds that remain in each fraction makes it difficult to determine the compound responsible for the observed effect taking weeks to months to perform. To achieve higher-resolution and faster effect-directed analysis, an androgen, estrogen and dioxin reporter assay and a luminescent mutagenicity assay were miniaturized and applied to samples fractionated on a newly developed UPLC/TOF-MS-based microfractionation setup. The applicability of the platform was demonstrated by analysis of water and passive sampler extracts from waste water treatment plant influent and effluent and from surface water. The use of microfractionation combined with indirect exposure of multiple assays from a single fractionation reported the effect of up to 228 fractions on up to eight different endpoints in parallel. Non-target analysis was performed on active fractions and revealed two confirmed estrogenic compounds and two candidate mutagens. The increase in resolution and throughput that parallel exposure and miniaturized bioassays provide, allows faster and more focused non-target analysis and accelerated identification of (novel) mutagens and endocrine disruptors using effect-directed analysis.

IN VITRO EFFECT-BASED TOXICITY PROFILING AND WATER MONITORING

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Integrated exposure and effect assessment of chemicals in the environment with state of the art effect-based bioanalysis tools is steadily increasing due to the needs to evaluate old & new pollutions with old & new outcome pathways in all kinds of environmental mixtures. Current focus in using such bioanalysis tools are for emerging pollutants such as POPs (dioxins, pesticides), endocrine disrupting chemicals (EDCs such as E2, EE2, estrone), and genotoxic compounds. We will give an overview about different international studies using human cell-based CALUX® tools for toxicity profiling and water monitoring. The latest results of international standardisation efforts (OECD TG 455, ISO 19040) for in vitro effect-based bioanalysis will be also presented.

We will report here about several mechanistic and toxicity based results of priority and hazardous pollutants of the water framework directives (WFD). We will use therefore a panel of standardized stable CALUX® human reporter gene cell lines to screen chemicals and complex mixtures as well as all kinds of waters on multiple toxicological pathways. The ultimate goal is a simple and fast method to accurately predict human and environmental hazard for unknown compounds and/or their complex mixtures. Effect-profiles on ca. 30 different CALUX® bioassays of approximately 200 reference compounds with known toxicological properties will be presented. Current global emphasis of EDC research lies on the measurement of estrogenic and androgenic compounds, while we will proof that also PAH-, (anti-) progesterone-, glucocorticoids- and obesogen- like activities are also relevant in toxicity profiling and for all kinds of water samples; such as WTP effluents (industrial and municipal), surface water and drinking water.

In the last two decades in vitro screening methods for EDCs such as our quantitative CALUX® bioassays have been evaluated and validated in several national (e.g. Descriset BE, LOES NL, Millenium Japan, CSIRO AUS, Be-Basic NL,) and international projects (such as ReProtect, Techneau and DEMEAU). Here also testing strategies have been developed for many additional relevant EDC endpoints (e.g. TR, ER, AR, PR, GR, PR, PPAR, genotox p53 and oxidative stress Nrf2 CALUX®) using HTPS robotic testing formats. In the current running follow up projects (e.g. EU ToxRisk, Be-Basic NL), the assessment of low but chronic exposure to EDCs, use of metabolic enzymatic systems (to simulate the HUMAN METABOLISM) and assessment of complex mixtures of EDCs to develop a suitable testing strategies (for e.g. NON-ANIMAL TESTING in relation to REACH/3R and drinking water testing) are evaluated, validated and applied in practise. The CALUX® toxicity profiling of in the WFD regulated substances and their effect-based water monitoring shows different activity profiles for different classes of compounds.

Such Bio-diagnostics now enables the screening of large sample numbers in a short period of time, while requiring only small amounts of substances and water material (ISO 19040-3 only 2 ml water). This makes the panel of CALUX® bioassays a promising fast, sensitive, efficient and economical tool for toxicological profiling with multiple areas of application, including incidental or routine monitoring and large water monitoring studies.

Screening Approach for Compounds in Surface- and Wastewater Samples Exhibiting Estrogenic Potentials

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The chemical analysis of contaminants and the assessment of unwanted biological effects in the aquatic environment are two sides of one coin. Both approaches provide complementary information about a given sample, but in many cases it is extremely challenging to combine results obtained by these two approaches to a consistent picture. The concept of the effect directed analysis (EDA) tries to bridge this knowledge gap between the compound- and the effect-universe by a combination of sample fractionation, biotesting and chemical analysis.

Due to the good separation efficiency, high performance liquid chromatography (HPLC) is commonly used for the fractionation of complex samples. However, because of a high number of samples that have to be tested after fractionation the HPLC-approach suffers from a limited sample throughput . The direct coupling of thin-layer chromatography (TLC) and a suborganismic bioassay was proposed as an alternative approach that might be useful for screening purposes in particular because the separated compounds are tested simultaneously on the surface of the TLC plate. As an example the planar Yeast Estrogen Screen (pYES) combines directly an effect specific bioassay for the biological detection of estrogen-receptor agonists with TLC.

Amounts below 1 pg E2 and EE2 can be detected and quantified by the pYES. This allows the quantification of E2 and EE2 in the range of 10 pg/l after a 1000-fold concentration of the sample using solid phase extraction. These low limits of quantification are sufficient for a reliable compliance check of EE2 according to the European Water Framework Directive (EC-WFD) with a discussed environmental quality standard of 35 pg/l.

For a validation, about 50 samples from either waste- or surface water were characterized by the pYES and the results were compared to data generated by a LC/MS-hr measurement. Correlation coefficients for waste water and surface water samples were > 0.9 indicating a satisfying accordance between both methods. The results measured for the analytes E2 and EE2 were consistent as well.

As for all specific bioassays the pYES can detect structurally uncharacterized compounds sharing the same mode of action, i.e. the activation of the estrogen receptor. Therefore, activity profiles of samples can be generated easily opening a wide range of different applications, e.g. source tracking of estrogenic compounds in the environment, and comparative assessment of alternative processes for wastewater treatment.

The pYES is comparable to classical chemical analysis allowing the detection and quantification of specific target compounds like EE2 and E2 but furthermore sheds light on the presence of other, yet unidentified compounds which might be of concern because of their endocrine potential. Like a classical bioassay it allows the quantification of the overall estrogenic potential of a sample but as well gives valuable information about individual effect contributions of the contaminants present in the sample.

EFFECTS OF EXPOSURE TO AN ANALOG OF THE JUVENILE HORMONE ON THE FRESHWATER AMPHIPOD *GAMMARUS FOSSARUM*

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Numerous studies have focused on the effects of vertebrate endocrine disruptors on arthropods. Nevertheless, given that endocrine systems have evolved differently across phylum, vertebrate endocrine disruptors could have little effects on arthropods. In the present study, we tested the effect of an exposure to an analog of the arthropod juvenile hormone (fenoxycarb) on the freshwater amphipod *Gammarus fossarum*. Fenoxycarb is a growth regulator insecticide used for controlling pest insect's populations (including lepidopteran, mosquito, cockroach and bug). Gammaridae have been widely used for laboratory toxicity testing, due to their sensitivity to toxic exposure, their abundance in European freshwater systems and the ease with which they can be handled. Moreover, since they contribute to the degradation of the organic matter and they serve as food for many macroinvertebrates, fishes and amphibians, they play a major role in the food web.

Two particularly sensitive life stages to endocrine disruption were investigated: the embryogenesis and the reproduction. During embryonic development, numerous morphogenetic factors and endocrine signaling pathways ensure the production of a target phenotype. Therefore, we tested the following hypothesis: an endocrine disruptor, by interfering with signaling pathways involved in morphogenesis, can cause an increase of the incidence of phenotypic defects in newborn individuals. Moreover, in the adult stage, female vitellogenesis is under endocrine regulation processes; we thus hypothesized that female exposure to an analog of the juvenile hormone could translate into a reduced reproduction success.

In newborn individuals from embryos that were exposed to fenoxycarb, we observed delayed hatching, eye pigmentation impairment and midgut tissue damages. Females that were previously exposed to fenoxycarb during ovogenesis exhibited a disturbed precopulatory behavior. Further studies are needed to determine whether these adverse effects were caused by endocrine disruption. In any case, the deleterious effects that were observed both in the embryo stage and in the adult stage could have long-term consequences on gammarid population dynamics.

The Combined Algae Assay - A Promising Tool for Water Quality Assessment

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Photosystem II (PSII)-inhibiting herbicides, such as diuron or terbuthylazine, are an important group of environmental pollutants. Consequently, six PSII inhibitors are priority substances under the EU Water Framework Directive (WFD). For these, Environmental Quality Standards (EQS) are determined and applied as a regulatory tool. In Switzerland, EQS have been derived for several PSII inhibitors which will soon be included in the Swiss Waters Protection Ordinance. PSII inhibitors are used as biocides as well as plant protection products and are regularly detected at environmentally relevant concentrations in Swiss surface waters. These compounds act specifically on primary producers such as algae and higher plants. Inhibition of photosynthesis has, aside of direct effects, also an impact on growth and the organisms' capability of coping with other (multiple) stressors. The combined algae assay – developed by Escher et al. (2008), building on work done by, for example, Schreiber et al. (2007) – has been used to measure effects of PSII inhibitors. We present an overview of the application of the assay in a regulatory context and examples from case studies.

The assay is conducted with single-cell green algae of the species *Raphidocelis subcapitata* and covers two endpoints: inhibition of photosynthetic activity and inhibition of growth rate. Inhibition of photosynthetic activity is a sum parameter for all herbicides with this mode of action and covers mixture effects. Inhibition of PSII occurs very fast (a few minutes after exposure of the algae to an active compound) and the endpoint is measured 2 h after test start. Effect data for environmental samples are expressed as equivalents of the reference compound diuron (diuron equivalent concentrations, DEQ). Inhibition of the 24 h growth rate – as an endpoint – is also caused by compounds which are not primarily considered as herbicides such as triclosan, various pharmaceuticals and metals.

The assay is simple and cost-effective, and its applicability and suitability for water quality assessment has been demonstrated in a number of studies. It allows for a robust analysis of mixtures, as DEQ concentrations correlate to a high degree with measured concentrations of PSII inhibiting herbicides such as atrazine, diuron, isoproturone, simazine, terbutryne and terbuthylazine. Currently, the assay is included in the test procedure to evaluate the suitability of wastewater for ozonation as tertiary treatment (Schindler Wildhaber et al. 2015) and in a first tier evaluation concept to assess water quality with ecotoxicological bioassays in Switzerland (Kienle et al. 2015). The assay has been applied in various large Swiss effluent and surface water monitoring campaigns (EcoImpact 13/14 and NAWA SPEZ 15/17). To further support the inclusion of the tool in a regulatory context we aim for an international standardisation (ISO) of the assay.

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"From toxicity assay to metabolomics analysis" - An integrated approach to assess the toxicity of three Benzotriazoles in zebrafish (*Danio rerio*) embryos

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The ever increasing contamination of the aquatic environment from xenobiotics has raised concerns in the scientific community and the regulatory authorities. Given the large number of xenobiotics, there is an important gap in the literature concerning their adverse effects on aquatic organisms. There is clear evidence that benzotriazoles (BTs) persist in aquatic systems, as they are measured in almost every surface water sample. Thus, it is urgent to evaluate their potentially toxic effects to aquatic organisms.

The objectives of this study were to assess in to what extent 1-H-benzotriazole (BT), 4-methyl-1-H-benzotriazole (4-MeBT) and 5-methyl-1-H-benzotriazole (5-MeBT) induce toxicity to zebrafish embryos. In addition, we evaluated the uptake and biotransformation of BTs by zebrafish and examined whether biotransformation data could be used complementary to the concentration of the parent compounds to interpret the induced toxicity. The final goal was to establish a wide-scope targeted metabolomics screening method to investigate the induced toxicity in a biochemical perspective and associate the observed toxicity/phenotype with changes in molecular level.

More specifically, the zebrafish embryo toxicity assay was used to calculate the LC50 values of BTs as well as to perform the morphological phenotyping. Concerning the biotransformation and the metabolomics experiment, 96-hours post fertilization (hpf) zebrafish were used. Samples were collected at 5 different time intervals, from 30 s up to 24 h post exposure (hpe), to examine the time profiles of the parent BTs, their biotransformation products (bio-TPs) and the endogenous metabolites of zebrafish. Extracts were analyzed by RPLC and HILIC methods, in both positive and negative ionization mode, to cover the widest possible range of polarities, using a LC-QTOF-HR-MS/MS instrument.

For the detection and identification of tentative bio-TPs, both suspect and non-target screening workflows have been applied. Both oxidative (hydroxylation) and conjugative (sulfation, glucuronidation) bio-TPs were identified. Moreover, biotransformation rate proved to be informative and correlated well with the observed toxicity. As regards the metabolomics part of the study, a database of over 600 endogenous metabolites (carboxylic acids, amines, nucleotides, mono- and disaccharides etc.) was established, covering a broad range of primary metabolism. The wide-scope targeted metabolomics method constitutes an alternative to the classic targeted methods, as it did not focus at a predefined set of metabolic pathways. The approach to cover a broad range of primary metabolism is hypothesis-generating rather than hypothesis-driven, as it enables to unravel the involvement of unexpected metabolic pathways.

The combination of morphological phenotyping information from acute toxicity test with internal concentration and biotransformation data from toxicokinetic experiment, in addition to the biochemical information from the wide-scope targeted metabolomics analysis, constitutes a high-throughput and integrated approach for the toxicity assessment.

APPLICATION OF NOVEL OMICS TOOLS FOR ZEBRAFISH (NEURO-) TOXICOLOGICAL RESEARCH

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In the last decade a variety of different omics technoligies were developed and are more and more applied in biological research. However, for toxicological studies, especially zebrafish, mostly transcriptomics is used. It has been shown by several studies that the gene expression patterns of zebrafish are altered after exposure to toxicants and that these alterations can be used to identify toxic mechanism. But genomic information alone cannot provide a complete picture of the physiological processes in an organism. Changes in the Proteome, Metabolome or Lipidome may provide a better understanding of the biological mechanisms, the structural and enzymatic changes in an organism and the impact of compounds on these mechanisms. Therefore, we apply novel State- of-the-Art Omics technologies to study transcriptomic, proteomic, metabolomic and lipidomic changes in zebrafish exposed to environmental relevant substances. The high sensetivity of our methods allows to analyze whole fish, tissues, e.g brain, of adult fish but also tissues of embryos or larvae. Our main focus lays especially on the mechanistic understanding of neurological processes and the impact of substances on the neurodevelopment. We studied proteomic changes in zebrafish larvae (5 dpf) after exposure to compounds using Isobaric Tandem Mass Tags® (TMT®) methods. The samples were pre-fractionated using basic RPLC and further analysed by LC-MS using MS^3 acquisition for accurate quantization. In total, 2,395 protein groups were identified, represented by 8,291 peptide sequences. Statistical analysis combined with pathway and gene ontology analysis revealed mechanistic overlaps between substances. To monitor metabolic changes of five neurotransmitter systems in parallel, including the dopaminergic/andrenergic, glutaminergic/ GABAnergic, serotoninergic, histaminergic and cholinergic systems, we developed a method based on hydrophilic interaction liquid chromatography coupled to tandem mass spectrometry. For the determination of monoamine neurotransmitters (MNTs) we developed another sensitive and fast analytical method using gas chromatography coupled to mass spectrometry. Our methods enable the quantification of neurotransmitters, their precursors and metabolites in whole zebrafish from the period of zygote to free swimming larvae 6 days post fertilization (dpf). We observed a developmental stage-dependent pattern, with clear differences between the first two days of development and the following days. Whereas the neurotransmitter levels steadily increased, the precursors showed a peak at 3 dpf. After exposure to several pesticides, significant differences in concentrations of neurotransmitters and precursors were observed. To study transcriptomic changes we designed a cost-effective Neurotox-array using multiplex qPCR. With this system we can screen 42 target genes in parallel. The genes are selected to be involved in important neurological and developmental processes. Toxicogenomic profils could be established for 39 compounds and could be successfully linked to behavioural effects. We also developed a lipidomic approach using LC coupled to high resolution time-of-flight mass spectrometry to study a large number of lipid classes, of which some are related to cell signalling and neuroinflammation.

Our research shows that the Transcriptome, Proteome, Metabolome and Lipidome of developing zebrafish are altered by exposure. Understanding these changes can help to gain new insights in the physiological changes in whole organisms after exposure. Such knowledge is important to improve our understanding of the link of exposure to chemicals and the development and progression of diseases.

Posters

INTEREST OF IN VITRO BIOASSAYS (YES/YAS) FOR THE SCREENING OF ENDOCRINE DISRUPTION IN SURFACE WATERS.

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This study is part of the BIODIEN project. This project aimed at conducting, for the first time, a screening campaign of endocrine disruptors (ED) in waters of Wallonia (groundwater, surface water and wastewater). Almost 200 substances were screened, including alkylphenols, phthalates, chlorophenols, perfluorates, PBDEs, PCBs, HAPs and pesticides.

In parallel with analytical methods, YES and YAS bioassays were conducted in order to quantify estrogenic and androgenic activities in surface waters. Antagonist activities were also evaluated. Over 71 river sampling points, estrogenic activity was detected and quantified at 53 sites and could reach levels up to 11.7 ng E2eq/l (mean: 2.1±1.6 ng E2eq/l). Androgenic activity was never detected. On the other hand, estrogenic and androgenic antagonist activities were detected in 41 % and 55 % of the river sites, respectively.

These activities are compared to the chemical results. The goal of this comparison is to evaluate whether bioassays can represent a valuable screening tool for endocrine disruptors/disruption in surface waterbodies. Indeed, chemical analyses can be expensive and, for some ED, limits of detection are high and can be well above Environment Quality Standards (as established through the Water Framework directive) levels. Bioassays, like YES/YAS, could be an appropriate alternative to chemical analyses for screening purposes.

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EFFECT MONITORING OF SELECTED EFFLUENTS IMPACTING SENSITIVE WATERBODIES AND USE OF BIOASSAYS TO ASSESS THE EFFICIENCY OF THE MANAGEMENT MEASURES.

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Bioassays are a meaningful way to assess effects of environmental samples and wastewaters on waterbodies. Since many years, studies are carried out with complementary tools in different rivers of the Meuse and Scheldt basins, upstream, downstream and in the effluent of selected industries and urban wastewater treatment plants. An effect active monitoring using bioassays was carried out, combining ecotoxicological and physico-chemical measurements. For this purpose, we use a battery of short term and chronic bioassays with the bacteria *Vibrio fischeri*, the alga *Pseudokirchneriella subcapitata*, the rotifer *Brachionus calyciflorus* and the microcrustacea *Daphnia magna*. Moreover, a yeast estrogen screen (YES) assay was conducted as an assessment tool to detect the presence of endocrine disrupting compounds. Priority List substances of the WFD and other pollutants discharged in significant quantities are also measured. The results of this monitoring show that bioassays are good diagnostic tools to determine the causes of poor ecological quality and to trace back to the source of contamination. They are an important “tool in the toolbox” for environmental management, helping to design appropriate management measures.

EcoImpact – Effects of Micropollutants from Wastewater Treatment Plants on Stream Ecosystems: Ecotoxicological and Chemical Evaluations in 24 Swiss Rivers

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Micropollutants are organic and inorganic substances, which occur in very low concentrations in surface waters. Even at these low concentrations, some of them can elicit effects on aquatic organisms. The aim of the project EcoImpact is to evaluate the effects of micropollutants originating from wastewater treatment plant (WWTP) effluents on stream ecosystems. The project combines chemical, microbiological, ecotoxicological and ecological evaluations in order to enable a comprehensive understanding of the processes in the river ecosystems. This paper focuses on a part of the project: the ecotoxicological and chemical evaluations.

Several measurement campaigns were conducted in 2013 and 2014: In 2013 a screening of 400 micropollutants as well as laboratory and field bioassays were performed. At selected sites, feeding activity of amphipods (*Gammarus fossarum*) as well as the reproduction of water flea (*Ceriodaphnia dubia*) were assessed. Additionally, effects on the photosynthesis and growth of single-celled green algae (*Pseudokirchnerielle subcapitata*), estrogenic activity (Yeast Estrogen Screen), and neurotoxic effects (acetylcholinesterase inhibition assay) were evaluated in all samples. The 2014 assessment programme consisted of the chemical evaluation of 60 substances as well as an assessment of estrogenic activity, algal toxicity and neurotoxic effects.

With regard to estrogenic activity and photosynthesis inhibition, the impact of the WWTP was obvious with mostly higher estradiol equivalent concentrations (EEQ) as well as diuron equivalent concentrations (DEQ) downstream than upstream of the WWTP. Similarly, a decreased amphipod feeding activity was detected in one of four sampling sites. A significantly decreased reproduction of *C. dubia* was detected in one of four sites as well.

The applied methods proved to be well suitable for a chemical and ecotoxicological assessment of river water quality. In general the contamination with estrogenic substances can be considered relatively low. The EEQs never exceeded the effect-based chronic environmental quality standard (Annual Average (AA) EQS) for 17β-Estradiol (0.4 ng/L). However, in a majority of the evaluated rivers, the DEQ exceeded the AA-EQS for diuron (20 ng/L), partly already upstream of the WWTP. Future data evaluation will include a comparison with the chemical and ecological data to complete the overall picture, and to draw further conclusions on the effects of micropollutants on stream ecosystems.

Ecotoxicological Monitoring of Photosystem II Inhibitors in Five Small Streams in Switzerland

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Small streams represent a major part of the Swiss river network, but so far information about pesticide pollution and pollution dynamics is limited. To investigate the situation in such small rivers, a special monitoring program (SPEZ) was conducted on five streams as part of the National Surface Water Quality Monitoring Network (NAWA) in spring and summer 2015. The aim of this project was to obtain knowledge about peak and baseline pesticide concentrations of over 250 different pesticides as well as ecotoxicological effects in surface water samples. Selected sampling sites were located in areas intensively used for agriculture with different types of crops (grain, vegetables, vineyards and orchards).

Twelve h composite water samples were taken continuously from April to August 2015 using automated sampling devices. Samples were extracted using solid phase extraction and subsequently examined for 250 pesticides and metabolites using HPLC-HRMS. To complement the chemical analysis with ecotoxicological effect data, one week composite samples were analysed for effects on two endpoints using the combined algae assay: inhibition of photosystem II activity and inhibition of growth rate. Toxic effects were expressed as diuron equivalent concentrations (DEQ) for photosystem II inhibition and as baseline toxicity equivalent concentrations (baseline-TEQ) for growth inhibition.

Photosynthesis inhibition was observed in 100% of the water samples, but at different levels. DEQs ranged between ca. 5 and 300 ng/L. Highest DEQs were found in June in areas with vineyards. In some cases high DEQs could be linked with rain events. During the sampling campaign longer periods without rain occurred whilst DEQs remained elevated. Consequently herbicides were introduced constantly to the streams even without rain events. The ecotoxicological effect data are compared to measured chemical concentrations and calculated risk quotients for plants.

Outlook: In 2017, between March and October, a similar program will be started in five streams using 3.5 days composite water samples. Chemical analysis of samples will be accompanied by different types of bioassay, e.g. the combined algae assay and Ceriodaphnia reproduction tests.

EFFECT-DIRECTED ANALYSIS OF WWTP EFFLUENTS BY LC × LC-TOF MS

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Effect-directed analysis (EDA) is a useful tool to identify environmental contaminants compounds in complex sample matrices. However, mass spectrometric identification in EDA is usually challenging, mainly due to severe matrix effects and ion suppression, partly as a result of limited separation power of the liquid chromatography based fractionation. In our study, for the first time a comprehensive two-dimensional liquid chromatography (LC × LC) based microfractionation system combined with parallel high resolution time of flight (HR-ToF) mass spectrometric detection and a high throughput acetylcholinesterase (AChE) assay was developed and applied for the analysis of emerging AChE inhibitors in wastewater treatment plant (WWTP) effluent.

Firstly, the analytical performance of the system was evaluated for carbamate pesticide mixture. The bioactivity observed in the four 96-well plates containing the fractions correlated excellently with the identity of the active compounds in these fractions. A C18 and pentafluorophenyl (PFP) stationary phase combination was selected for the two-dimensional separation and fractionation in four 96-well plates. Then the effluent sample was fractionated by LC × LC in four 96-well plates and the orthogonality was evaluated using two different methods. High orthogonality (0.937 or 0.945, respectively) was achieved. Subsequently, the solvent was evaporated from the wells and the high throughput AChE assay was applied to the total 384 wells, to determine the active fractions. Finally, several environmental contaminants (e.g. pharmaceuticals and metabolites) were identified in the active fractions using the ESI (+)-ToF MS data acquired in parallel, according to the detected accurate masses and isotopic patterns. Three AChE inhibitors (tiapride, amisulpride, and lamotrigine), used as antipsychotic medicines were identified and confirmed by two-dimensional retention alignment as well as their AChE inhibition activity.

Comparing with traditional one-dimensional liquid chromatography, the peak capacity of LC × LC was greatly enhanced. The improved separation power also reduced the matrix effect, which led to an easier identification of environmental contaminants using high resolution mass spectrometry. Therefore, LCxLC-ToF-MS in combination with an in vitro high throughput assay is a valuable tool for comprehensive characterization of toxicity and identity of environmental contaminants in complex environmental samples.

Effects of weathering on PFASs used in durable water repellence of textiles

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Per- and polyfluoroalkyl substances (PFASs) are used in textiles for their oil and water repellent properties. Because PFASs with long perfluorinated chains have been shown to be persistent in the environment, bioaccumulative and (eco)toxic, the textile industry is phasing-out the long-chain PFASs and is replacing those compounds with alternative chemistries to deliver the desired durable water and soil repellent (DWR) effect. Those alternative chemistries can be divided in three main groups: fluorocarbon-based, silicon-based and hydrocarbon-based polymers. During our research in the SUPFES (Substitution in practice of prioritised fluorinated compounds for textile applications) project, the alternative DWRs are assessed to (i) their structural properties and connected performance, (ii) loss and degradation processes resulting in diffuse environmental emissions, and (iii) hazard profile for the emitted substances. As part of SUPFES the influence of weathering conditions on the different types of DWR treatments are assessed. Here the effect of weathering on the PFASs concentrations in nine textiles of outdoor clothing is presented. To assess the effect of weathering on the type of PFAS and their concentration in outdoor clothing during the life time of the clothing, nine textile samples of different types of outdoor clothing were exposed to elevated UV radiation, humidity, and temperature in an aging device for 300 h, which is equivalent to the life time of the outdoor clothing. The textile samples were, before and after aging, extracted with methanol and analysed by LS-MS/MS with a validated extraction and analyses method for perfluoroalkyl acids (PFAAs) in textiles
Weather conditions, like sunlight, high temperature, or humidity have an effect on PFASs used in DWR of outdoor clothing. In some samples the concentration of PFAAs increased and PFAA not present in the original textiles were formed during exposure of weather conditions. More research is needed to clarify the origin of the PFAAs and to determine the transformation route.

EFFECT-ASSESSMENT OF WASTEWATER BY MOLECULAR BIOMARKERS IN TROUT

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Preference: Poster presentationen

The identification and monitoring of chemical effects in the environment is dominantly based on analytical chemistry and toxicity data for a few model species. Accounting for the variety of species and their different responses to environmental stressors is still a big challenge for ecotoxicological risk assessment. mRNA expression analysis of selected biomarker genes is a promising approach for field monitoring of non-model organisms because it can capture a wide spectrum of responses of organisms to chemical exposure. We established a biomarker gene set for brown trout (*Salmo trutta*) to assess the effects of micropollutants released by wastewater effluents. The biomarker set consisted of 20-25 genes which reflect different cellular stress responses like general stress, oxidative stress, endocrine disruption or xenobiotic-biotransformation. The transcriptional regulation of these genes was measured in liver tissue samples of wild brown trout caught downstream and upstream of different wastewater treatment plants (WWTP) in Switzerland. Furthermore, we investigated the effect of advanced wastewater treatment techniques, such as ozonation and activated carbon filtration to reduce the input of micropollutants in the environment, by collecting samples one year before and after the WWTP upgrade. Results from different sampling sites before WWTP upgrading showed that mRNA levels are site dependent. Fish taken downstream generally express a different transcriptional regulation pattern compared to fish from the upstream, indicating that fish downstream are exposed to a higher level of environmental stressors. For example, the metal (Metallothionein B), endocrine disruption (Vitellogenin) or biotransformation (Cyp3a) sensitive genes were found to be significantly up-regulated in fish from the downstream location compared to upstream of the WWTP. Results implicated a higher content of metals, potential estrogen-mimics or different pharmaceuticals downstream of the WWTP. Indeed, chemical analysis and further bioassays confirmed high concentrations and effects of such compounds in the water from downstream compared to upstream. Partially, concentrations were above the threshold value for surface water. Results from sampling campaigns one year after WWTP upgrading clearly showed a reduction of the differences in biomarker expression in fish from up- and downstream locations, indicating improved water quality due to new wastewater treatment techniques. Our data demonstrates that quantifying expression levels of selected biomarker genes is a very sensitive technique which allows an assessment of exposure and related effects of chemicals. It appears that our method is a promising screening assay for assessing surface water quality and pollutant responses in resident fish.